


16

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INTERNATIONAL PRELIMINARY EXAMINATION REPORT **PCT**

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference <b>91641</b>	<b>FOR FURTHER ACTION</b>	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416).
International application No. <b>PCT/AU99/00306</b>	International filing date (day/month/year) <b>23 April 1999</b>	Priority Date (day/month/year) <b>23 April 1998</b>
International Patent Classification (IPC) or national classification and IPC <b>Int. Cl. <sup>7</sup> C12Q 1/68, 1/48; C12N 15/54</b>		
Applicant <b>COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANISATION et al</b>		

1.	This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.
2.	This REPORT consists of a total of <b>3</b> sheets, including this cover sheet. <input type="checkbox"/> This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).  These annexes consist of a total of <b>52</b> sheet(s).
3.	This report contains indications relating to the following items:  I <input checked="" type="checkbox"/> Basis of the report II <input type="checkbox"/> Priority III <input type="checkbox"/> Non-establishment of opinion with regard to novelty, inventive step and industrial applicability IV <input type="checkbox"/> Lack of unity of invention V <input checked="" type="checkbox"/> Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement VI <input type="checkbox"/> Certain documents cited VII <input type="checkbox"/> Certain defects in the international application VIII <input type="checkbox"/> Certain observations on the international application

Date of submission of the demand <b>11 November 1999</b>	Date of completion of the report <b>4 July 2000</b>
Name and mailing address of the IPEA/AU <b>AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaaustralia.gov.au Facsimile No. (02) 6285 3929</b>	Authorized Officer  <b>JULIE CAIRNDUFF</b> Telephone No. (02) 6283 2545

**I. Basis of the report**

1. With regard to the **elements** of the international application:\*
- ☐ the international application as originally filed.
- ☒ the description, pages **1 to 4, 12 to 44**, as originally filed,  
pages , filed with the demand,  
pages **5 to 11**, received on **26 June 2000** with the letter of **26 June 2000**
- ☒ the claims, pages , as originally filed,  
pages , as amended (together with any statement) under Article 19,  
pages , filed with the demand,  
pages **62 to 72**, received on **26 June 2000** with the letter of **26 June 2000**
- ☒ the drawings, pages , as originally filed,  
pages , filed with the demand,  
pages **1/17 to 17/17**, received on **30 June 1999** with the letter of **30 June 1999**
- ☒ the sequence listing part of the description:  
pages , as originally filed  
pages , filed with the demand  
pages **1/17 to 17/17**, received on **30 June 1999** with the letter of **30 June 1999**
2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.  
These elements were available or furnished to this Authority in the following language which is:
- ☐ the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).
3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, was on the basis of the sequence listing:
- ☐ contained in the international application in written form.
- ☒ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished
4. ☒ The amendments have resulted in the cancellation of:
- ☐ the description, pages **45-61**
- ☐ the claims, Nos.
- ☐ the drawings, sheets/fig.
5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).\*\*

\* Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).

\*\* Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report

**V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement****1. Statement**

Novelty (N)	Claims 1-50	YES
	Claims	NO
Inventive step (IS)	Claims 1-50	YES
	Claims	NO
Industrial applicability (IA)	Claims 1-50	YES
	Claims	NO

**2. Citations and explanations (Rule 70.7)**Citations

D1: AU 31341/95 (THE JOHN HOPKINS UNIVERSITY SCHOOL OF MEDICINE) 1 February 1991

D2: Lee, W.H. et al. (1997) Cancer Epidemiology, Biomarkers and Prevention, volume 6, pp. 443-450

D3: Jhaveri, M.S. et al. (27 March 1998) Gene, volume 210, number 1, pp. 1-7

D4: Lee W.H. et al. (1994) Proceedings of the National Academy of Sciences USA, volume 91, pp. 11733-11737

D5: Frommer M. et al. (1992) Proceedings of the National Academy of Sciences USA, volume 89, number 5, pp. 1827-1831

Novelty and Inventive Step

Claims 1 to 50 are considered novel and inventive when compared to any one of D1 to D5. None of the citations disclose a method to identify abnormal methylation of cytosine within the glutathione-S-transferase (GST) Pi gene or its regulatory flanking sequences defined by (and inclusive of) CpG sites -43 to +55, using a selective amplification technique. Furthermore none of D1 to D5 identify specific sequences that may be used in the exemplified method. As a result claims 1 to 50 are novel and inventive.

Industrial Applicability

Claims 1 to 46 exhibit industrial applicability.

said site or sites at which abnormal cytosine methylation occurs is/are methylated, and

(iii) determining the presence of amplified DNA,

wherein the amplifying step (ii) is used to amplify a target region of the GST-Pi gene and/or its regulatory flanking sequences defined by (and inclusive of) CpG sites -43 to +55.

Since the amplification is designed to only amplify the target region if the said site or sites at which abnormal cytosine methylation (i.e. as compared to the corresponding site or sites of DNA from subjects without the disease or condition being assayed) occurs is/are methylated, the presence of amplified DNA will be indicative of the disease or condition in the subject from which the isolated DNA has been obtained. The assay thereby provides a means for diagnosing or prognosing the disease or condition in a subject.

The step of isolating DNA may be conducted in accordance with standard protocols. The DNA may be isolated from any suitable body sample, such as cells from tissue (fresh or fixed samples), blood (including serum and plasma), semen, urine, lymph or bone marrow. For some types of body samples, particularly fluid samples such as blood, semen, urine and lymph, it may be preferred to firstly subject the sample to a process to enrich the concentration of a certain cell type (e.g. prostate cells). One suitable process for enrichment involves the separation of required cells through the use of cell-specific antibodies coupled to magnetic beads and a magnetic cell separation device.

Prior to the amplifying step, the isolated DNA is preferably treated such that unmethylated cytosines are converted to uracil or another nucleotide capable of forming a base pair with adenine while methylated cytosines are unchanged or are converted to a nucleotide capable of forming a base pair with guanine. This treatment permits the design of primers which enable the selective amplification of the target region if the said site or sites at which abnormal cytosine methylation occurs is/are methylated.

Preferably, following treatment and amplification of the isolated DNA, a test is performed to verify that unmethylated cytosines have been efficiently converted to uracil or another nucleotide capable of forming a base pair with adenine, and that methylated cytosines have remained unchanged or efficiently converted to another nucleotide capable of forming a base pair with guanine.

Preferably, the treatment of the isolated DNA involves reacting the isolated DNA with bisulphite in accordance with standard protocols. As will be clear from the above discussion of bisulphite treatment, unmethylated cytosines will be converted to uracil whereas methylated cytosines will be unchanged. Verification that unmethylated cytosines have been converted to uracil and that methylated cytosines have remained unchanged may be achieved by;

(i) restricting an aliquot of the treated and amplified DNA with a suitable restriction enzyme(s) which recognise a restriction site(s) generated by or resistant to the bisulphite treatment, and

(ii) assessing the restriction fragment pattern by electrophoresis.

Alternatively, verification may be achieved by differential hybridisation using specific oligonucleotides targeted to regions of the treated DNA where unmethylated cytosines would have been converted to uracil and methylated cytosines would have remained unchanged.

The amplifying step may involve polymerase chain reaction (PCR) amplification, ligase chain reaction amplification (20) and others (21).

Preferably, the amplifying step is conducted in accordance with standard protocols for PCR amplification, in which case, the reactants will typically be suitable primers, dNTPs and a thermostable DNA polymerase, and the conditions will be cycles of varying temperatures and durations to effect alternating denaturation of strand duplexes, annealing of primers (e.g. under high stringency conditions) and subsequent DNA synthesis.

To achieve selective PCR amplification with bisulphite-treated DNA, primers and conditions may be used to discriminate between a target region including a site or sites of abnormal cytosine methylation and a target region where there is no site or sites of abnormal cytosine methylation. Thus, for  
5 amplification only of a target region where the said site or sites at which abnormal cytosine methylation occurs is/are methylated, the primers used to anneal to the bisulphite-treated DNA (i.e. reverse primers) will include a guanine nucleotide(s) at a site(s) at which it will form a base pair with a methylated cytosine(s). Such primers will form a mismatch if the target  
10 region in the isolated DNA has unmethylated cytosine nucleotide(s) (which would have been converted to uracil by the bisulphite treatment) at the site or sites at which abnormal cytosine methylation occurs. The primers used for annealing to the opposite strand (i.e. the forward primers) will include a cytosine nucleotide(s) at any site(s) corresponding to site(s) of methylated  
15 cytosine in the bisulphite-treated DNA.

Preferably, the primers used for the PCR amplification are of 12 to 30 nucleotides in length and are designed to anneal to a sequence within the target region that includes two to four cytosine nucleotides that are abnormally methylated in the DNA of a subject with the disease or condition  
20 being assayed. In addition, the primers preferably include a terminal nucleotide that will form a base pair with a cytosine nucleotide (reverse primer), or the guanine nucleotide opposite (forward primer), that is abnormally methylated in the DNA of a subject with the disease or condition being assayed.

25 The step of amplifying is used to amplify a target region within the GST-Pi gene and/or its regulatory flanking sequences. The regulatory flanking sequences may be regarded as the flanking sequences 5' and 3' of the GST-Pi gene which include the elements that regulate, either alone or in combination with another like element, expression of the GST-Pi gene.

In particular, the step of amplifying is used to amplify a target region within the region of the GST-Pi gene and its regulatory flanking sequences defined by (and inclusive of) CpG sites -43 to +55 (wherein the numbering of the CpG sites is relative to the transcription start site). The numbering and position of CpG sites is shown in Figure 1.

The step of determining the presence of amplified DNA may be conducted in accordance with standard protocols. One convenient method involves visualisation of a band(s) corresponding to amplified DNA, following gel electrophoresis.

Preferably, the disease or condition to be assayed is selected from cancers, especially hormone dependent cancers such as prostate cancer, breast cancer and cervical cancer, and liver cancer.

For the diagnosis or prognosis of prostate cancer, the step of amplifying preferably amplifies a target region within the region of the GST-Pi gene and its regulatory flanking sequences defined by (and inclusive of) CpG sites -43 to +53, more preferably, -43 to +10. However, within these target regions it is believed that there are CpG sites which show variability in methylation status in prostate cancer or are methylated in other tissues. Thus, for the target region defined by (and inclusive of) CpG sites -43 to +10, it is preferred that the primers used for amplification be designed so as to minimise (i.e. by use of redundant primers or by avoidance of the sites) the influence of CpG sites -36, -32, -23, -20, -19, -14 and a polymorphic region covering site -33. Further, for DNA isolated from cells other than from prostate tissue (e.g. blood), it is preferred that the primers used be designated to amplify a target region that does not include the region of the GST-Pi gene and its regulatory flanking sequences defined by (and inclusive of) CpG sites -7 to +7, or, more preferably, -13 to +8, since this may lead to false positives. Further preferred target regions, therefore, are within the region of the GST-Pi gene and its regulatory flanking sequences defined by (and inclusive of) CpG sites -43 to -14, -43 to -8, +9 to +53 and +1 to +53.

Suitable primer pairs for the diagnosis or prognosis of prostate cancer, include those consisting of a forward and reverse primer selected from each of the following groups:

Forward Primers (i.e. anneal to the 5' end of the target region)

- |    |                               |                |
|----|-------------------------------|----------------|
| 5  | CGCGAGGTTTTCGTTGGAGTTTCGTCGTC | (SEQ ID NO: 1) |
|    | CGTTATTAGTGAGTACGCGCGGTTC     | (SEQ ID NO: 2) |
|    | YGGTTTTAGGGAATTTTTTTTCGC      | (SEQ ID NO: 3) |
|    | YGGYGYGTTAGTTYGTTGYGTATATTTC  | (SEQ ID NO: 4) |
|    | GGGAATTTTTTTTCGCGATGTTTYGGCGC | (SEQ ID NO: 5) |
| 10 | TTTTTAGGGGGTTYGGAGCGTTTC      | (SEQ ID NO: 6) |
|    | GGTAGGTTGYGTTTATCGC           | (SEQ ID NO: 7) |

Reverse Primers (i.e. anneal to the extension of the forward primer)

- |    |                                  |                 |
|----|----------------------------------|-----------------|
|    | TCCCATCCCTCCCGAAACGCTCCG         | (SEQ ID NO: 8)  |
|    | GAAACGCTCCGAACCCCTAAAAACCGCTAACG | (SEQ ID NO: 9)  |
| 15 | CRCCCTAAAATCCCCRAAATCRCCGCG      | (SEQ ID NO: 10) |
|    | ACCCCRACRACCRCTACACCCRAACGTCG    | (SEQ ID NO: 11) |
|    | CTCTTCTAAAAAATCCCRCAACTCCCGCCG   | (SEQ ID NO: 12) |
|    | AAAACRCCCTAAAATCCCCGAAATCGCCG    | (SEQ ID NO: 13) |
|    | AACTCCCRCCGACCCCAACCCGACGACCG    | (SEQ ID NO: 14) |
| 20 | AAAAATTCRAATCTCTCCGAATAAACG      | (SEQ ID NO: 15) |
|    | AAAAACCRAAATAAAAACCACACGACG      | (SEQ ID NO: 16) |

wherein Y is C, T or, preferably, a mixture thereof, and R is A, G or, preferably, a mixture thereof.

- For the diagnosis or prognosis of liver cancer, the step of amplifying preferably amplifies a target region within the region of the GST-Pi gene and its regulatory flanking sequences defined by (and inclusive of) CpG sites -43 to -14 and/or +9 to +53. However, within these target regions it is believed that there are CpG sites which show variability in methylation status in liver cancer or are methylated in other tissues. Thus, for the target region defined by (and inclusive of) CpG sites -43 to -14, it is preferred that the primers used



for amplification be designed so as to minimise (i.e. by use of redundant primers or by avoidance of the sites) the influence of CpG sites -36, -32, -23, -20, -19, -14 and a polymorphic region covering site -33.

5 It will be appreciated by persons skilled in the art, that a site or sites of abnormal cytosine methylation within the above identified target regions of the GST-Pi gene and/or its regulatory flanking sequences, could be detected for the purposes of diagnosing or prognosing a disease or condition (particularly, prostate cancer and/or liver cancer) by methods which do not involve selective amplification. For instance, oligonucleotide/polynucleotide  
10 probes could be designed for use in hybridisation studies (e.g. Southern blotting) with bisulphite-treated DNA which, under appropriate conditions of stringency, selectively hybridise only to DNA which includes a site or sites of abnormal methylation of cytosine(s). Alternatively, an appropriately selected informative restriction enzyme(s) could be used to produce restriction  
15 fragment patterns that distinguish between DNA which does and does not include a site or sites of abnormal methylation of cytosine(s).

Thus, in a second aspect, the present invention provides a diagnostic or prognostic assay for a disease or condition in a subject said disease or condition characterised by abnormal methylation of cytosine at a site or sites  
20 within the glutathione-S-transferase (GST) Pi gene and/or its regulatory flanking sequences, wherein said assay comprises the steps of;

- (i) isolating DNA from said subject, and
- (ii) determining the presence of abnormal methylation of cytosine at a site or sites within the region of the GST-Pi gene and/or its regulatory flanking  
25 sequences defined by (and inclusive of) CpG sites -43 to +55.

The step of isolating DNA may be conducted as described above in relation to the assay of the first aspect.

Preferably, the region of the GST-Pi gene and its regulatory flanking sequences within which the presence of methylated cytosine(s) at a site or  
30 sites is determined is selected from the regions defined by (and inclusive of)

CpG sites -43 to +53, -43 to +10, -43 to -14, +9 to +53 and +1 to +53.

However, within these regions, it is preferred that certain sites (namely, CpG sites, -36, -33, -32, -23, -20, -19, and -14) be avoided as the site or sites at which, for the purpose of the assay, the presence of abnormal methylation of cytosine is determined.

Where the determination step is to involve selective hybridisation of oligonucleotide/polynucleotide/peptide-nucleic acid (PNA) probes, prior to the determination step, the isolated DNA is preferably treated (e.g. with bisulphite) such that unmethylated cytosines are converted to uracil or another nucleotide capable of forming a base pair with adenine while methylated cytosines are unchanged or are converted to a nucleotide capable of forming a base pair with guanine. This treatment permits the design of probes which allow for selective hybridisation to a target region including a site or sites of abnormal methylation of cytosine.

In a third aspect, the present invention provides a primer or probe (sequence shown in the 5' to 3' direction) comprising a nucleotide sequence selected from the group consisting of:

	CGCGAGGTTTTCGTTGGAGTTTCGTCGTC	(SEQ ID NO: 1)
	CGTTATTAGTGAGTACGCCGCGTTC	(SEQ ID NO: 2)
20	YGGTTTTAGGGAATTTTTTTTCGC	(SEQ ID NO: 3)
	YGGYGYGTTAGTTYGTTGYGTATATTC	(SEQ ID NO: 4)
	GGGAATTTTTTTTCGCGATGTTYGGCGC	(SEQ ID NO: 5)
	TTTTTAGGGGGTTYGGAGCGTTTC	(SEQ ID NO: 6)
	GGTAGGTTGYGTTTATCGC	(SEQ ID NO: 7)
25	AAAAATTCRAATCTCTCCGAATAAACG	(SEQ ID NO: 8)
	AAAAACCRAAATAAAAACCACACGACG	(SEQ ID NO: 9)
	TCCCATCCCTCCCCGAAACGCTCCG	(SEQ ID NO: 10)
	GAAACGCTCCGAACCCCTAAAAACCGCTAACG	(SEQ ID NO: 11)
	CRCCCTAAAATCCCCRAAATCRCCGCG	(SEQ ID NO: 12)
30	ACCCCRACRACCRCTACACCCRAACGTCG	(SEQ ID NO: 13)

**Claims:**

1. A diagnostic or prognostic assay for a disease or condition in a subject,  
5 said disease or condition characterised by abnormal methylation of cytosine  
at a site or sites within the glutathione-S-transferase (GST) Pi gene and/or its  
regulatory flanking sequences, wherein said assay comprises the steps of;  
(i) isolating DNA from said subject,  
(ii) exposing said isolated DNA to reactants and conditions for the  
10 amplification of a target region of the GST-Pi gene and/or its regulatory  
flanking sequences which includes a site or sites at which abnormal cytosine  
methylation characteristic of the disease or condition occurs, the  
amplification being selective in that it only amplifies the target region if the  
said site or sites at which abnormal cytosine methylation occurs is/are  
15 methylated, and  
(iii) determining the presence of amplified DNA,  
wherein the amplifying step (ii) is used to amplify a target region within the  
region of the GST-Pi gene and/or its regulatory flanking sequences defined by  
(and inclusive of) CpG sites -43 to +55.  
20
2. An assay according to claim 1, wherein prior to the amplifying step,  
the isolated DNA is treated such that unmethylated cytosines are converted  
to uracil or another nucleotide capable of forming a base pair with adenine  
while methylated cytosines are unchanged or are converted to a nucleotide  
25 capable of forming a base pair with guanine.
3. As assay according to any one of the preceding claims, wherein the  
amplifying step involves polymerase chain reaction (PCR) amplification.
- 30 4. An assay according to claim 3, wherein said PCR amplification utilises  
a reverse primer including guanine at at least one site whereby, upon the

reverse primer annealing to the treated DNA, said guanine will either form a base pair with a methylated cytosine (or another nucleotide to which the methylated cytosine has been converted through said treatment) if present, or will form a mismatch with uracil (or another nucleotide to which unmethylated cytosine has been converted through said treatment).

5. An assay according to claim 4, wherein said PCR amplification utilises a forward primer including cytosine at at least one site(s) corresponding to cytosine nucleotides that are abnormally methylated in the DNA of a subject with the disease or condition being assayed.

6. An assay according to claim 5, wherein the primers are of 12 to 30 nucleotides in length.

7. An assay according to claim 6, wherein the primers are selected so as to anneal to a sequence within the target region that includes two to four cytosine nucleotides that are abnormally methylated in the isolated DNA of a subject with the disease or condition being assayed.

8. An assay according to claim 2, wherein the treatment of the isolated DNA involves reacting the isolated DNA with bisulphite.

9. As assay according to claim 8, wherein the amplifying step involves polymerase chain reaction (PCR) amplification.

10. An assay according to claim 9, wherein said PCR amplification utilises a reverse primer including guanine at at least one site whereby, upon the reverse primer annealing to the treated DNA, said guanine will either form a base pair with a methylated cytosine if present, or will form a mismatch with uracil.

11. An assay according to claim 10, wherein said PCR amplification utilises a forward primer including cytosine at at least one site(s) corresponding to cytosine nucleotides that are abnormally methylated in the isolated DNA of a subject with the disease or condition being assayed.
12. An assay according to claim 11, wherein the primers are of 12 to 30 nucleotides in length.
13. An assay according to claim 12, wherein the primers are selected so as to anneal to a sequence within the target region that includes two to four cytosine nucleotides that are abnormally methylated in the DNA of a subject with the disease or condition being assayed.
14. An assay according to any one of the preceding claims, wherein said DNA is isolated from cells from tissue, blood (including serum and plasma), semen, urine, lymph or bone marrow.
15. An assay according to any one of the preceding claims, wherein the disease or condition to be assayed is selected from cancers.
16. An assay according to claim 15, wherein the disease or condition to be assayed is selected from prostate cancer, breast cancer, cervical cancer and liver cancer.
17. An assay according to claim 16, wherein the disease or condition to be assayed is prostate cancer.
18. An assay according to claim 17, wherein the amplifying step is used to amplify a target region within the region of the GST-Pi gene and its

regulatory flanking sequences defined by (and inclusive of) CpG sites -43 to +53.

19. An assay according to claim 17, wherein the amplifying step is used to  
5 amplify a target region within the region of the GST-Pi gene and its  
regulatory flanking sequences defined by (and inclusive of) CpG sites -43 to  
+10.

20. An assay according to claim 17, wherein the amplifying step is used to  
10 amplify a target region within the region of the GST-Pi gene and its  
regulatory flanking sequences defined by (and inclusive of) CpG sites -43 to  
-14.

21. An assay according to claim 17, wherein the amplifying step is used to  
15 amplify a target region within the region of the GST-Pi gene and its  
regulatory flanking sequences defined by (and inclusive of) CpG sites -43 to  
-8.

22. An assay according to any one of the preceding claims wherein the  
20 target region excludes any or all of the CpG sites -36, -32, -23, -20, -19 and  
-14.

23. An assay according to any one of claims 5 to 21, wherein if either or  
both of the reverse or forward primers anneal to a sequence within the target  
25 region that includes any or all of CpG sites -36, -32, -23, -20, -19 and -14, then  
said PCR amplification further utilises equivalent reverse and/or forward  
primers including a redundant nucleotide(s) at the position(s) within their  
sequence(s) corresponding to cytosine or methylated cytosine of CpG sites -  
36, -32, -23, -20, -19 and -14.

30

24. An assay according to claim 17, wherein the amplifying step is used to amplify a target region within the region of the GST-Pi gene and its regulatory flanking sequences defined by (and inclusive of) CpG sites +9 to +53.

5

25. An assay according to claim 17, wherein the amplifying step is used to amplify a target region within the region of the GST-Pi gene and its regulatory flanking sequences defined by (and inclusive of) CpG sites +1 to +53.

10

26. An assay according to claim 17, wherein the amplifying step involves PCR amplification using primer pairs consisting of a forward and reverse primer selected from each of the following groups:

Forward Primers

15	CGCGAGGTTTTCGTTGGAGTTTCGTCGTC	(SEQ ID NO: 1)
	CGTTATTAGTGAGTACGCGCGGTTC	(SEQ ID NO: 2)
	YGGTTTTAGGGAATTTTTTTTCGC	(SEQ ID NO: 3)
	YGGYGYGTTAGTTYGTTGYGTATATTTC	(SEQ ID NO: 4)
	GGAATTTTTTTTCGCGATGTTYGGCGC	(SEQ ID NO: 5)
20	TTTTTAGGGGGTTYGGAGCGTTTC	(SEQ ID NO: 6)
	GGTAGGTTGYGTTTATCGC	(SEQ ID NO: 7)

Reverse Primers

	TCCCATCCCTCCCCGAAACGCTCCG	(SEQ ID NO: 8)
	GAAACGCTCCGAACCCCTAAAAACCGCTAACG	(SEQ ID NO: 9)
25	CRCCCTAAAATCCCCRAAATCRCCGCG	(SEQ ID NO: 10)
	ACCCCRACRACCRCTACACCCRAACGTCG	(SEQ ID NO: 11)
	CTCTTCTAAAAAATCCCRCAACTCCCGCCG	(SEQ ID NO: 12)
	AAAACRCCCTAAAATCCCCGAAATCGCCG	(SEQ ID NO: 13)
	AACTCCCRCCGACCCCAACCCCGACGACCG	(SEQ ID NO: 14)
30	AAAAATTCRAATCTCTCCGAATAAACG	(SEQ ID NO: 15)

AAAAACCRAAATAAAAACCCACACGACG (SEQ ID NO: 16),  
wherein Y is C, T or a mixture thereof, and R is A, G or a mixture thereof.

27. An assay according to claim 17, wherein the amplifying step involves  
5 PCR amplification using primer pairs consisting of a forward and reverse  
primer selected from each of the following groups:

Forward Primers

CGCGAGGTTTTCGTTGGAGTTTCGTCGTC (SEQ ID NO: 1)

CGTTATTAGTGAGTACGCGCGGTTC (SEQ ID NO: 2)

10 Reverse Primers

TCCCATCCCTCCCCGAAACGCTCCG (SEQ ID NO: 8)

GAAACGCTCCGAACCCCCTAAAAACCGCTAACG (SEQ ID NO: 9).

28. An assay according to claim 17, wherein the amplifying step involves  
15 PCR amplification using primer pairs consisting of a forward and reverse  
primer selected from each of the following groups:

Forward Primers

YGGTTTTAGGGAATTTTTTTTCGC (SEQ ID NO: 3)

YGGYGYGTTAGTTYGTTGYGTATATTC (SEQ ID NO: 4)

20 GGGAAATTTTTTTTCGCGATGTTTYGGCGC (SEQ ID NO: 5)

Reverse Primers

CRCCCTAAAATCCCCRAAATCRCCGCG (SEQ ID NO: 10)

ACCCCRACRACCRCTACACCCRAACGTCG (SEQ ID NO: 11)

CTCTTCTAAAAAATCCCRCAACTCCCGCCG (SEQ ID NO: 12)

25 AAAACRCCCTAAAATCCCCGAAATCGCCG (SEQ ID NO: 13)

AACTCCCRCCGACCCCAACCCCGACGACCG (SEQ ID NO: 14),

wherein Y is C, T or a mixture thereof and R is A, G or a mixture thereof.

29. An assay according to claim 17, wherein the amplifying step involves  
30 PCR amplification using primer pairs consisting of a forward and reverse



primer selected from each of the following groups:

Forward Primers

TTTTTAGGGGGTTYGGAGCGTTTC (SEQ ID NO: 6)

GGTAGGTTGYGTTTATCGC (SEQ ID NO: 7)

5

Reverse Primers

AAAAATTCRAATCTCTCCGAATAAACG (SEQ ID NO: 15)

AAAAACCRAAATAAAAACCACACGACG (SEQ ID NO: 16),

wherein Y is C, T or a mixture thereof, and R is A, G or a mixture thereof.

10 30. An assay according to claim 16, wherein the disease or condition to be assayed is liver cancer.

15 31. An assay according to claim 30, wherein the amplifying step is used to amplify a target region within the region of the GST-Pi gene and its regulatory flanking sequences defined by (and inclusive of) CpG sites -43 to -14.

20 32. An assay according to claim 31, wherein the target region excludes any or all of the CpG sites -36, -32, -23, -20, -19, and -14.

25 33. An assay according to claim 30, wherein if either or both of the reverse or forward primers anneal to a sequence within the target region that includes any or all of CpG sites -36, -32, -23, -20, -19 and -14, then said PCR amplification further utilises equivalent reverse and/or forward primers including a redundant nucleotide(s) at the position(s) within their sequence(s) corresponding to cytosine or methylated cytosine of CpG sites -36, -32, -23, -20, -19 and -14.

30 34. An assay according to claim 30, wherein the amplifying step is used to amplify a target region within the region of the GST-Pi gene and its

regulatory flanking sequences defined by (and inclusive of) CpG sites +9 to +53.

35. A diagnostic or prognostic assay for a disease or condition in a subject  
5 said disease or condition characterised by abnormal methylation of cytosine  
at a site or sites within the glutathione-S-transferase (GST) Pi gene and/or its  
regulatory flanking sequences, wherein said assay comprises the steps of;  
(i) isolating DNA from said subject, and  
(ii) determining the presence of abnormal methylation of cytosine at a site  
10 or sites within the region of the GST-Pi gene and/or its regulatory flanking  
sequences defined by (and inclusive of) CpG sites -43 to +55.

36. An assay according to claim 35, wherein the region of the GST-Pi gene  
and its regulatory flanking sequences within which the presence of  
15 methylated cytosine(s) at a site or sites is determined is selected from the  
regions defined by (and inclusive of) CpG sites -43 to +53, -43 to +10, -43 to  
-14, +9 to +53 and +1 to +53.

37. An assay according to claim 35 or 36, wherein the said region of the  
20 GST-Pi gene and its regulatory flanking sequences excludes any or all of the  
CpG sites -36, -32, -23, -20, -19 and -14.

38. An assay according to claim 36, wherein the region of the GST-Pi gene  
and its regulatory flanking sequences within which the presence of  
25 methylated cytosine(s) at a site or sites is determined is the region defined by  
(and inclusive of) CpG sites +9 to +53.

39. An assay according to claim 36, wherein the region of the GST-Pi gene  
and its regulatory flanking sequences within which the presence of

methyated cytosine(s) at a site or sites is determined is the region defined by (and inclusive of) CpG sites +1 to +53.

5 40. An assay according to any one of claims 35 to 39, wherein prior to the determination step, the isolated DNA is treated such that unmethylated cytosines are converted to uracil or another nucleotide capable of forming a base pair with adenine while methylated cytosines are unchanged or are converted to a nucleotide capable of forming a base pair with guanine.

10 41. An assay according to claim 40, wherein the treatment of the isolated DNA involves reacting the isolated DNA with bisulphite.

15 42. An assay according to any one of claims 35 to 41, wherein the determination step involves selective hybridisation of oligonucleotide/polynucleotide/peptide-nucleic acid (PNA) probe(s).

20 43. An assay according to claim 42, wherein if the probe(s) hybridise to a sequence within the target region that includes any or all of CpG sites -36, -32, -23, -20, -19 and -14, then said selective hybridisations further utilises equivalent probe(s) including a redundant nucleotide(s) at the position(s) within their sequence(s) corresponding to cytosine or methylated cytosine of CpG sites -36, -32, -23, -20, -19 and -14.

25 44. An assay according to any one of claims 35 to 43, wherein said DNA is isolated from cells from tissue, blood (including serum and plasma), semen, urine, lymph or bone marrow.

30 45. An assay according to any one of claims 35 to 43, wherein the disease or condition to be assayed is selected from cancers.

46. An assay according to claim 45, wherein the disease or condition to be assayed is selected from prostate cancer, breast cancer, cervical cancer and liver cancer.

5 47. An assay according to claim 46, wherein the disease or condition to be assayed is prostate cancer.

48. An assay according to claim 46, wherein the disease or condition to be assayed is liver cancer.

10

49. A primer or probe comprising a nucleotide sequence selected from the group consisting of:

	CGCGAGGTTTTCGTTGGAGTTTCGTCGTC	(SEQ ID NO: 1)
	CGTTATTAGTGAGTACGCGCGGTTC	(SEQ ID NO: 2)
15	YGGTTTITAGGGAATTTTTTTTCGC	(SEQ ID NO: 3)
	YGGYGYGTTAGTTYGTTGYGTATATTTC	(SEQ ID NO: 4)
	GGGAATTTTTTTTCGCGATGTTTYGGCGC	(SEQ ID NO: 5)
	TTTTTAGGGGGTTYGGAGCGTTTC	(SEQ ID NO: 6)
	GGTAGGTTGYGTTTATCGC	(SEQ ID NO: 7)
20	AAAAATTCRAATCTCTCCGAATAAACG	(SEQ ID NO: 8)
	AAAAACCRAAATAAAAACCACACGACG	(SEQ ID NO: 9)
	TCCCATCCCTCCCCGAAACGCTCCG	(SEQ ID NO: 10)
	GAAACGCTCCGAACCCCCTAAAAACCGCTAACG	(SEQ ID NO: 11)
	CRCCCTAAAATCCCCRAAATCRCCGCG	(SEQ ID NO: 12)
25	ACCCCRACRACCRCTACACCCCRACGTCG	(SEQ ID NO: 13)
	CTCTTCTAAAAAATCCCRCAACTCCCGCCG	(SEQ ID NO: 14)
	AAAACRCCCTAAAATCCCCGAAATCGCCG	(SEQ ID NO: 15)
	AACTCCCRCCGACCCCAACCCCGACGACCG,	(SEQ ID NO: 16),

wherein Y is a mixture of C and T, and R is a mixture of A and G.

30

50. A probe comprising a nucleotide sequence selected from the group consisting of:

AAACCTAAAAAATAAACAAACAA (SEQ ID NO: 17)

GGGCCTAGGGAGTAAACAGACAG (SEQ ID NO: 18)

5 CCTTTCCCTCTTTCCCARRTCCCCA (SEQ ID NO: 19)

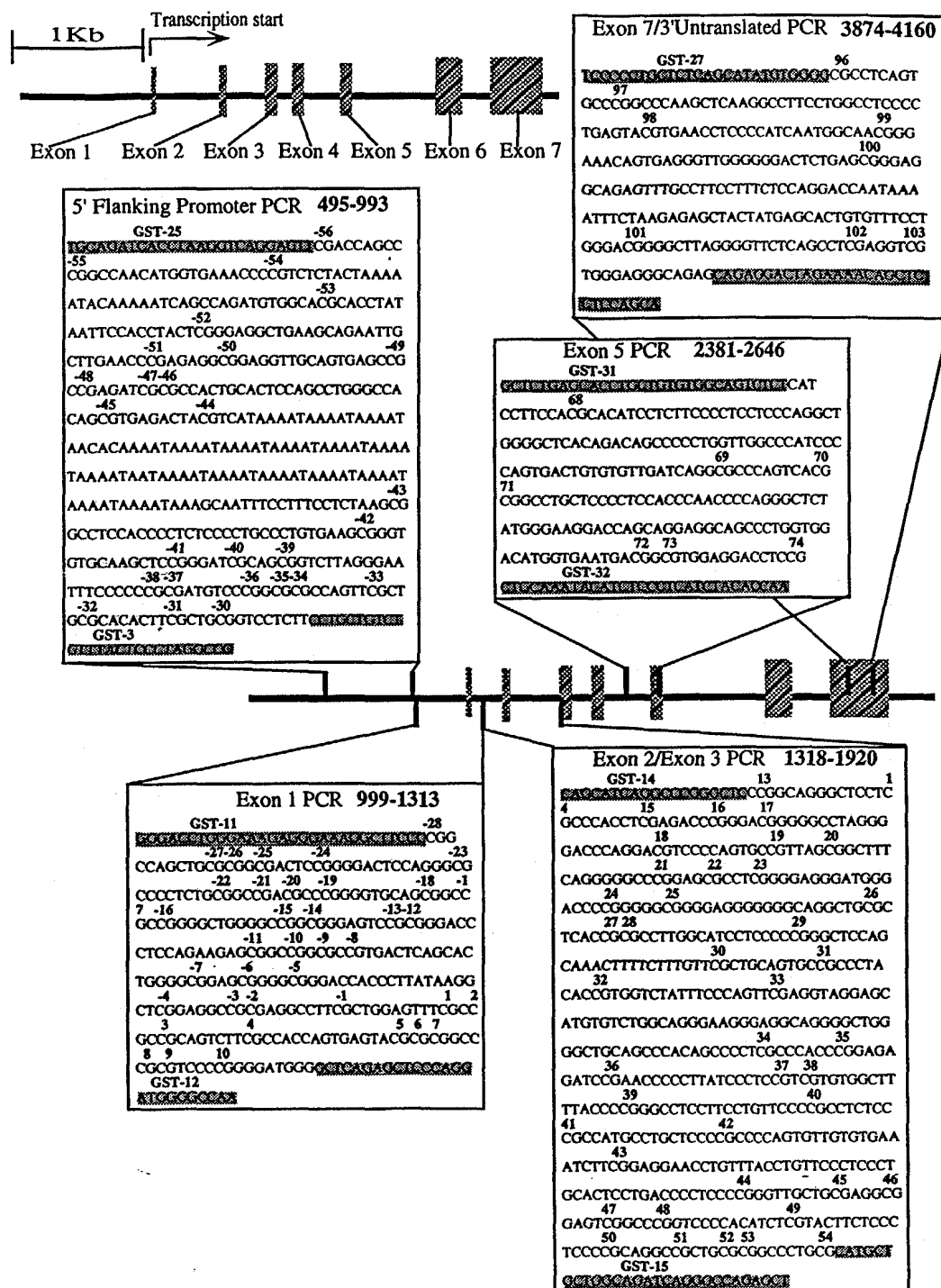
TTTGGTATTTTTTTTCGGGTTTTAG (SEQ ID NO: 20)

CTTGGCATCCTCCCCCGGGCTCCAG (SEQ ID NO: 21)

GGYAGGGAAGGGAGGYAGGGGYTGGG (SEQ ID NO: 22).

10

1 / 17



**Figure 2**  
**Upstream Region of Differential Methylation in Prostate Cancer**

-42  
 -43  
 -41 -40 -39 -38-37 -36-35-34 -33 -32  
 GTGTGCAAGC TCCGGGATCG CAGCGGTCTT AGGGAATTTT CCCCCGCGAT GTCCCGGCGC GCCAGTTCGC TCGCACACT -275  
 GTGTGTAAGT TTTGGGATTG TAGTGGTTTT AGGGAATTTT TTTTGTGAT GTTTGTGTGT GTAGTTTGT TGTGTATATT B-U  
 GTGTGTAAGT TTCGGGATCG TAGCGGTTTT AGGGAATTTT TTTTCGCGAT GTTTCGGCGC GTTAGTTTCGT TCGGTATATT B-M  
 CGPS-5 YGGTTTT AGGGAATTTT TTTTCG>CGPS-6 YGGYGY GTTAGTTYGT TGYGTATATT  
 CGPS-11 GGGAAATTTT TTTTCGCGAT GTTTYGGCGC>  
 -31 -30 -29 -28  
 TCGTGCCTG CCTCTTCTG CTGTCTGTTT ACTCCCTAGG CCCCCTGGG GACCTGGGAA AGAGGGAAAG GCTTCCCCCG -195  
 TGTGTGCTG TTTTCTTTTG TGTGTGTTT ATTTTGTAG TTTTGTGGG GATTGGGAA AGAGGGAAAG GTTTTTCGG B-U  
 TCGTGCCTG TTTTCTTTTG TGTGTGTTT ATTTTGTAG TTTCTTGGG GTTTGGGAA AGAGGGAAAG GTTTTTCGG B-M  
 TC>  
 -27-26-25 -24 -23 -22 -21 -20 -19 -18 -17 -16  
 CCAGCTGCGC GCGACTCCG GGGACTCCAG GCGCCCCCTC TCGGGCCGAC GCCCGGGTG CAGCGGCCGC CGGGCTGGG -115  
 TTAGTTGTGT GGTGATTTG GGGATTTAG GGTGTTTTT TGTGGTTGAT GTTTGGGGTG TAGTGGTTGT TGGGGTTGG B-U  
 TTAGTTGCGC GCGGATTCG GGGATTTAG GCGTTTTTT TCGGTTCCG GTTTCGGGTG TAGCGGTCTG CGGGGTGGG B-M  
 <GCG CCRCCTAAARC CCCTAAATC CCRC CGPS-7 < GCTG CAARCCCCAC ATCRCCARCA RCCCCA CGPS-8  
 <G CCGCTAAAGC CCCTAAATC CCRC AAAA CGPS-12 <GCCARCA GCCCCAACCC

3/17

Figure 2 (Continued)

-15 -14 -13-12 -11 -10 -9 -8 -7 -6 -5  
GCCGGCGGGA GTCCGCGGGA CCTCCAGAA GAGCGGCCGG CGCGTGACT CAGCACTGGG GCGGAGCGGG GCGGGACCCAC -35  
GTTGGTGGGA GTTGTGGGA TTTTGTAGAA GAGTGGTTGG TGTGTGATT TAGTATTGGG GTGGAGTGGG GTGGGATTAT B-U  
GTCGGCGGGA GTTCGCGGGA TTTTGTAGAA GAGCGGTCGG CGTCGTGATT TAGTATTGGG GCGGAGCGGG GCGGGATTAT B-M

<GCCGCCCT CAARCRCCCT AAAAATCTT CTC CGPS-9  
CAGCCRCCT CAA CGPS-13

-4 -3 -2 -1 > 1 2 3 4 5 6 7  
CCTTATAAGG CTCGGAGGCC GCGAGGCCTT CGCTGGAGTT TCGCCGCCGC AGTCTTCGCC ACCAGTGAGT ACGCGCGGCC +46  
TTTATAAGG TTTGGAGGTT GTGAGGTTTT TGTGGAGTT TGTGTGTTGT AGTTTTTGT ATTAGTGAGT ATGTGTGGTT B-U  
TTTATAAGG TTCGGAGGTC GCGAGGTTTT CGTTGGAGTT TCGTCGTCGT AGTTTTCGTT ATTAGTGAGT ACGCGCGGTT B-M

CGPS-1 C GCGAGGTTTT CGTTGGAGTT TCGTCGTC> CGPS-2 CGTT ATTAGTGAGT ACGCGCGGTT

8 9 10  
CGCGTCCCCG GGGATGGGC TCAGAGCTCC CAGCATGGGG CCAA +90  
TGTGTTTTTG GGGATGGGT TTAGAGTTTT TAGTATGGGG TTAA B-U  
CGCGTTTTTCG GGGATGGGT TTAGAGTTTT TAGTATGGGG TTAA B-M

C>



4/17

Figure 3A Methylation Status of Individual Sites in the GST-Pi Gene

site	LN	Du	PC3	PC3 M	PC3 MM	2AN	BN CN	2AC	BC	CC	DC	XC	WC	Pr
								4+4	3+3	2+3	2+2	3+4	3+3	
-28	++++	-	+	++	++	-	-	-	++	+	++	++++	++	-
-27	++++	-	+	++	++	-	-	-	+	+	++	++++	+	-
-26	++++	-	+	++	++	-	-	-	+	+	++	++++	++	-
-25	++++	-	+	++	++	-	-	-	+	++	++	++++	++	-
-24	++++	-	+	++	++	-	-	-	+	++	++	++++	+++	-
-23	++++	-	+	+	+	-	-	-	++	++	++	-	+++	-
-22	++++	-	++	+	+	-	-	-	++	+	++	-	+++	-
-21	++++	-	+	-	-	-	-	-	++	++	+++	+++	++	-
-20	++++	-	-	-	-	-	-	-	++	++	++	-	+	-
-19	++++	-	++	-	-	-	-	-	+	++	++	+	+	-
-18	++++	-	++	+	++	-	-	-	++	++	++	+++	++	-
-17	++++	-	+	+	++	-	-	-	++	++	++	+++	+++	-
-16	++++	-	++	+	++	-	-	-	+	++	+++	+++	+++	-
-15	++++	-	+	+	++	-	-	-	++	++	+++	+++	+++	-
-14	++++	-	-	+	+	-	-	-	+	++	++	-	+	-
-13	++++	-	+	+	+++	-	-	-	B	B	B	++	+	-
-12	++++	-	+	++	++	-	-	-	B	B	B	+++	+	-
-11	++++	-	++	++	++	-	-	-	+	++	++	+++	+++	-
-10	++++	-	++	++	++	-	-	-	++	++	+++	+++	+++	-
-9	++++	-	++	++	++	-	-	-	++	++	++	+++	+++	-
-8	++++	-	++	+	++	-	-	-	++	+	+++	+++	+++	-
-7	++++	-	++	++	++	-	-	-	++	++	+++	+++	+++	-
-6	++++	-	++	++	++	-	-	-	++	++	+++	+++	+++	-
-5	++++	-	++	++	+++	-	-	-	++	++	+++	+++	+++	-
-4	++++	-	++	++	+++	-	-	-	++	++	+++	+++	+++	-
-3	++++	-	++	++	++	-	-	-	++	++	+++	+++	+++	-
-2	++++	-	++	++	B	-	-	-	+++	++	+++	+++	+++	-
-1	++++	-	++	+	B	-	-	-	++	++	++	+++	++	-

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5/17

Figure 3A (cont'd)

1	+++	-	++	B	B	-	-	-	-	++	++	+++	+	-
2	+++	-	++	B	B	-	-	-	-	++	++	+++	+	-
3	+++	-	++	B	B	-	-	-	-	++	++	+++	+	-
4	+++	-	++	B	B	-	-	-	-	++	++	+++	+	-
5	+++	-	+++	B	B	-	-	-	-	B	++	+++	+	-
6	+++	-	+++	B	B	-	-	-	-	B	++	+++	+	-
7	+++	-	+++	B	B	-	-	-	-	B	++	+++	+	-
8	+++	-	++	B	B	-	-	-	-	B	++	+++	+	-
9	+++	-	++	B	B	-	-	-	-	B	++	+++	+	-
10	+++	-	++	B	B	-	-	-	-	B	++	+++	+	-
11														
12														
13	+++		+	+++	+++		-	-	-	+++	+++	+++	+	-
14	+++		+	+++	+++		-	-	-	+++	+++	+++	+	-
15	+++		+	+++	+++		-	-	-	+++	+++	+++	+	-
16	+++		+	+++	+++		-	-	-	+++	+++	+++	+	-
17	+++		+	++	++		-	-	-	++	+++	+++	+	-
18	+++		+	++	+		-	-	-	++	+++	+++	+	-
19	+++		+	+	+		-	-	-	++	+++	+++	+	-
20	+++		+	+	+		-	-	-	++	+++	+++	+	-
21	+++		B	+	+		-	-	-	++	+++	+++	+	-
22	+++		B	+	+		-	-	-	++	+++	+++	+	-
23	+++		B	+	+		-	-	-	++	+++	+++	+	-
24	+++		B	-	-		-	-	-	++	+++	+++	+	-
25	-		B	+	+		-	-	-	++	+++	+++	+	-
26	+++		B	++	B		-	-	-	+++	+++	+++	+	-
27	+++		B	+++	B		-	-	-	+++	+++	+++	+	-
28	+++		B	+++	B		-	-	-	+++	+++	+++	+	-
29	+++		B	+++	B		-	-	-	B	+++	+++	+	-
30	+++		B	B	B		-	-	-	+++	B	+++	+	-
31	+++		B	B	B		-	-	-	+++	B	+++	+	-
32	B		B	B	B		-	-	-	+++	B	+++	+	-
33	+++		B	+++	B		-	-	-	+++	+++	+++	+	-

6/17

Figure 3A (cont'd)

[illegible]

### Figure 3B Methylation Status of Individual Sites in the GST-Pi Gene

[illegible]

[illegible]

FIGURE 3B (cont'd)

9/17

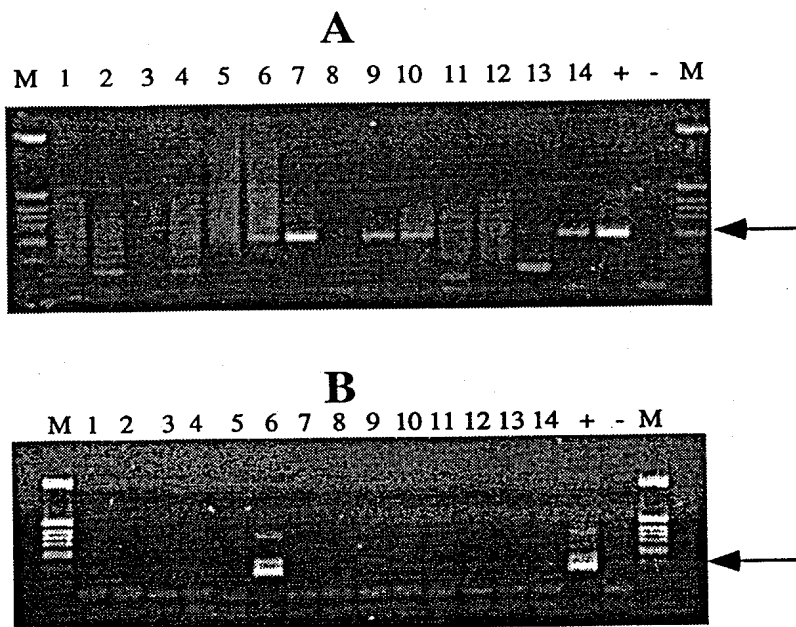
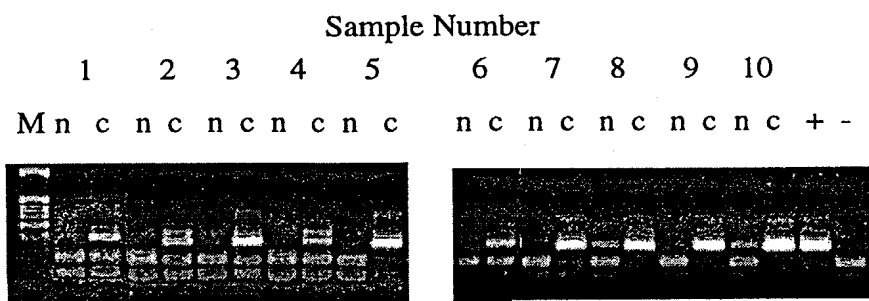


FIGURE 4A

10/17

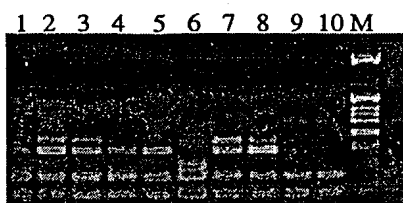


Sample	Tissue	Gleason	% Methylation Non CG rich PCR
1	Normal	N/A	-
	Cancer	3+3	++++
2	Normal	N/A	-
	Cancer	3+5	++
3	Normal	N/A	-
	Cancer	3+3	++
4	Normal	N/A	-
	Cancer	3+5	-
5	Normal	N/A	-
	Cancer	2+2	++
6	Normal	N/A	-
	Cancer	3+3	-
7	Normal	N/A	-
	Cancer	2+3	++
8	Normal	N/A	-
	Cancer	3+3	++
9	Normal	N/A	-
	Cancer	2+3	++++
10	Normal	N/A	-
	Cancer	?	++

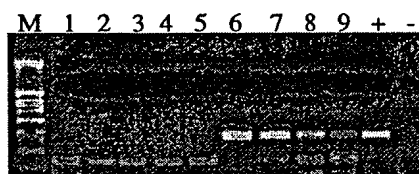
FIGURE 4B

11/17

**A**



**B**



**C**

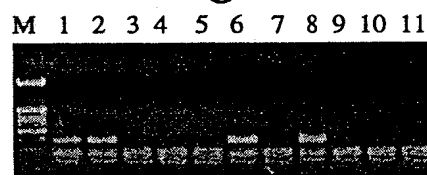


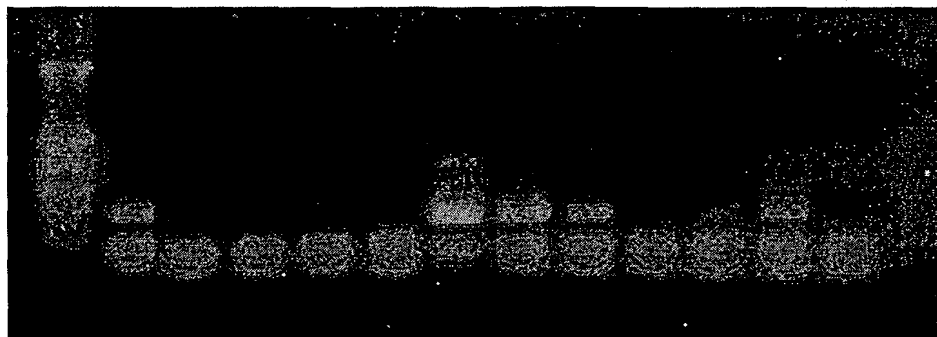
FIGURE 4C



12/17

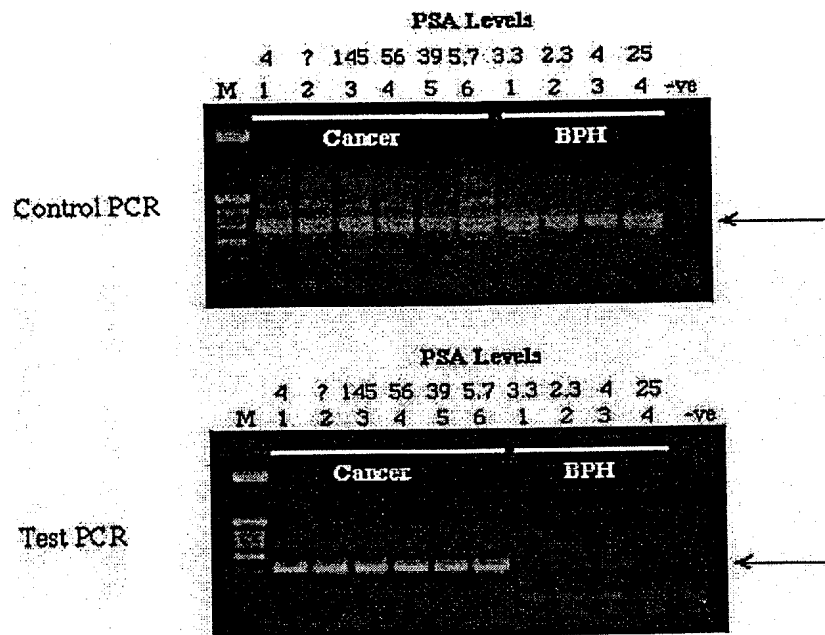
**Figure 5**

M L D P N N C C C N N N -



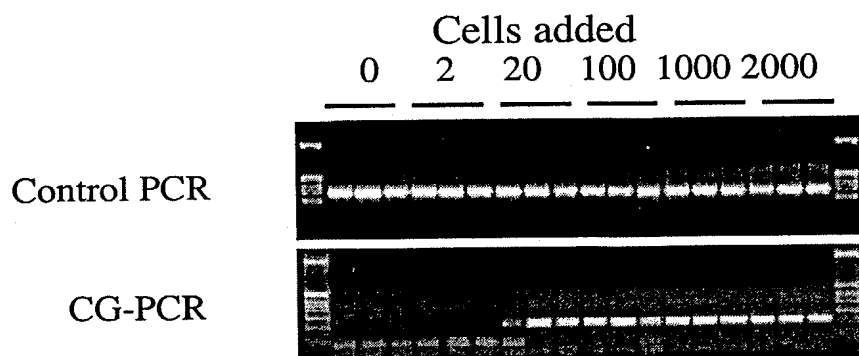
13/17

Figure 6

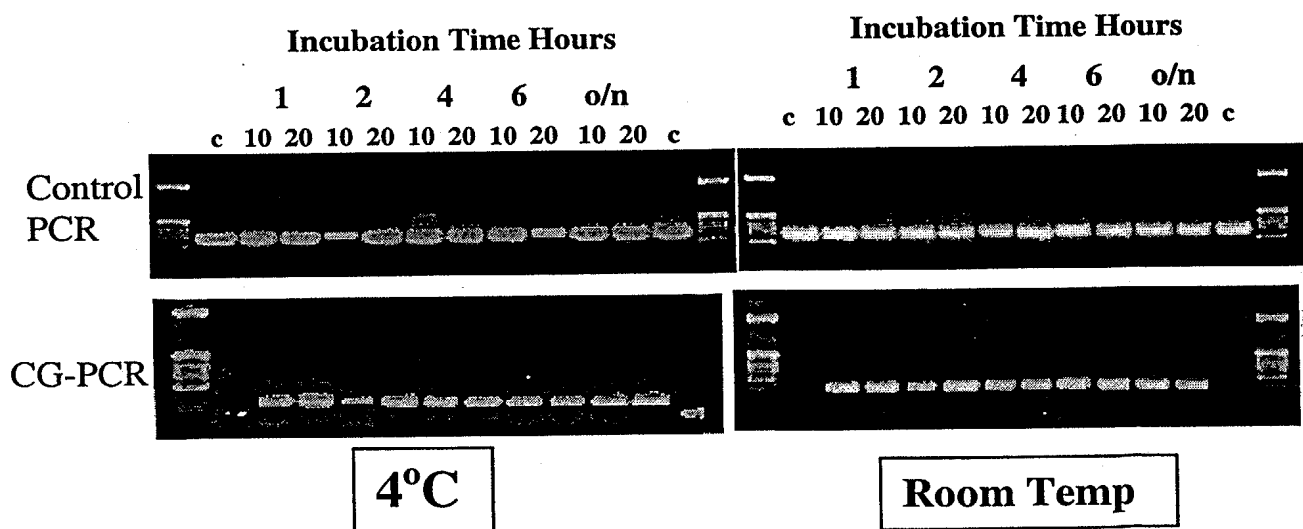


14/17

**Figure 7A**

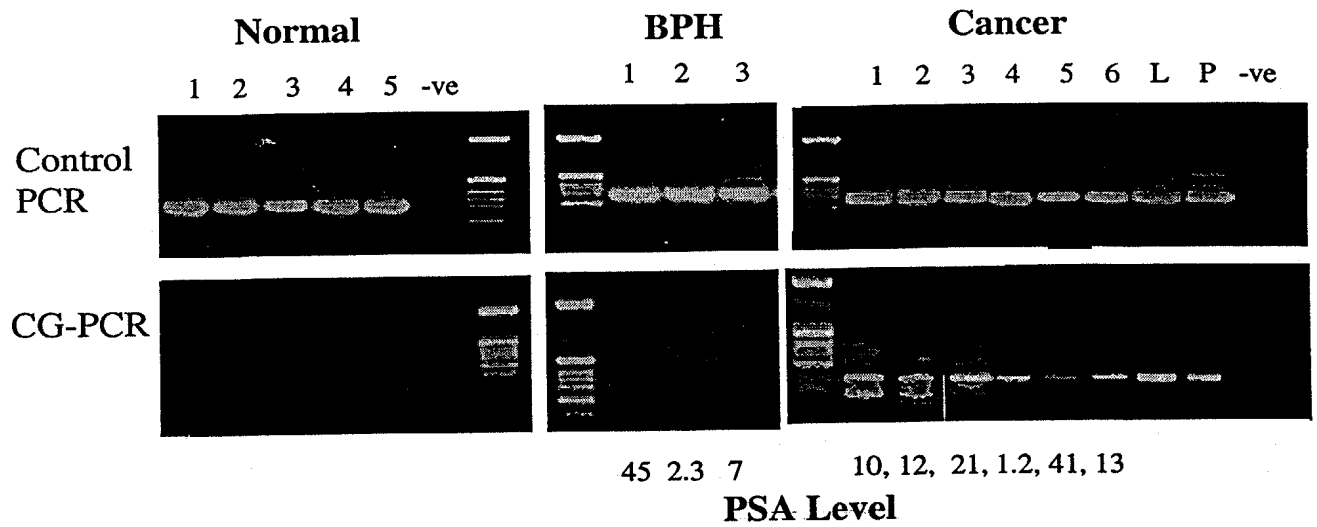


**Figure 7B**



15/17

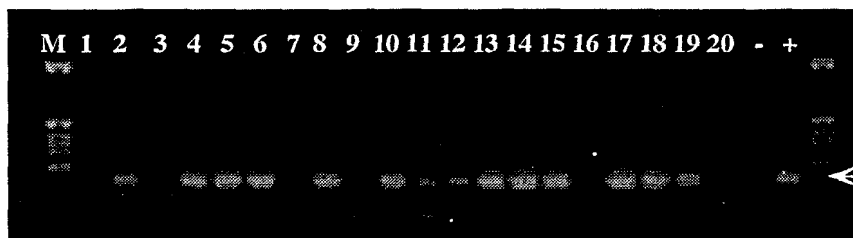
**Figure 8**



16/17

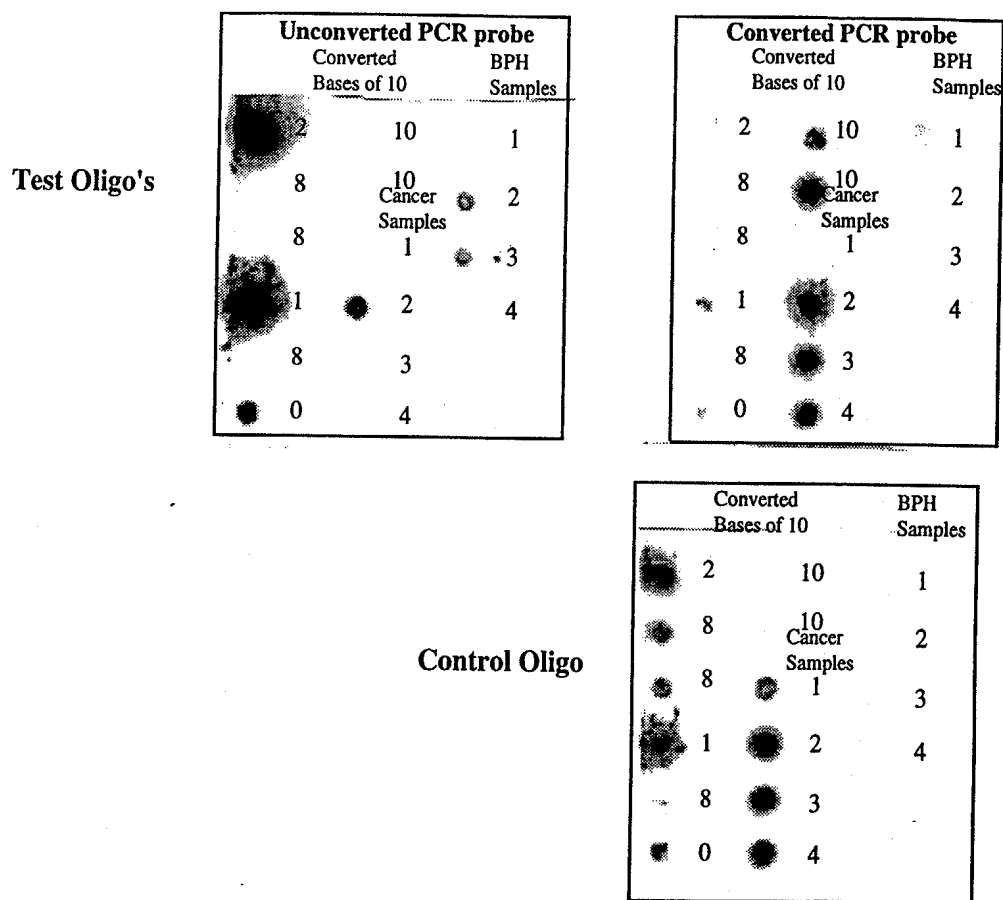
**Figure 9**

**Liver Cancer Tissue DNA extracts**



17/17

Figure 10



1/17

Sequence Listings:

Applicant: Commonwealth Scientific and Industrial Research  
Organisation

Title: Diagnostic assay

Prior Application Number: PP3129

Prior Application Filing Date: 1998-04-23

Number of SEQ ID NOs: 59

Software: PatentIn Ver. 2.1

SEQ ID NO: 1

Length: 29

Type: DNA

Organism: Homo sapiens

Sequence: 1

cgcgagggttt tcgttggagt ttcgtcgtc

29

SEQ ID NO: 2

Length: 25

Type: DNA

Organism: Homo sapiens

Sequence: 2

cgttattagt gagtacgcgc gggttc

25

SEQ ID NO: 3

Length: 24

Type: DNA

Organism: Homo sapiens

2/17

Sequence: 3  
yggtttttagg gaattttttt tcgc 24

SEQ ID NO: 4  
Length: 28  
Type: DNA  
Organism: Homo sapiens

Sequence: 4  
ygggygygta gtttggttgyg tatatttc 28

SEQ ID NO: 5  
Length: 29  
Type: DNA  
Organism: Homo sapiens

Sequence: 5  
gggaattttt tttcgcgatg ttttyggcgc 29

SEQ ID NO: 6  
Length: 24  
Type: DNA  
Organism: Homo sapiens

Sequence: 6  
tttttagggg gtttyggagcg tttc 24

SEQ ID NO: 7  
Length: 19  
Type: DNA  
Organism: Homo sapiens

Sequence: 7  
ggtaggttgy gtttatcgc 19

SEQ ID NO: 8



3/17

Length: 27

Type: DNA

Organism: Homo sapiens

Sequence: 8

aaaaattcra atctctccga ataaacg

27

SEQ ID NO: 9

Length: 27

Type: DNA

Organism: Homo sapiens

Sequence: 9

aaaaaccraa ataaaaacca cagcagc

27

SEQ ID NO: 10

Length: 25

Type: DNA

Organism: Homo sapiens

Sequence: 10

tcccatccct ccccgaaacg ctccg

25

SEQ ID NO: 11

Length: 33

Type: DNA

Organism: Homo sapiens

Sequence: 11

gaaacgctcc gaaccccta aaaaccgcta acg

33

SEQ ID NO: 12

Length: 27

Type: DNA

Organism: Homo sapiens

4/17

Sequence: 12

crcctaaaa tcccraaat crccgcg

27

SEQ ID NO: 13

Length: 30

Type: DNA

Organism: Homo sapiens

Sequence: 13

acccracra ccrctacacc ccraacgtcg

30

SEQ ID NO: 14

Length: 31

Type: DNA

Organism: Homo sapiens

Sequence: 14

ctcttctaaa aaatcccr cr aactcccgcc g

31

SEQ ID NO: 15

Length: 29

Type: DNA

Organism: Homo sapiens

Sequence: 15

aaaacrcct aaaatccccg aaatcgccg

29

SEQ ID NO: 16

Length: 30

Type: DNA

Organism: Homo sapiens

Sequence: 16

aactcccrcc gacccaacc ccgacgaccg

30

SEQ ID NO: 17

5/17

Length: 23

Type: DNA

Organism: Artificial Sequence

Feature:

Other Information: Description of Artificial

Sequence:Oligonucleotide

which binds bisulfite-converted human GST-Pi gene

Sequence: 17

aaacctaataa aataaaca aa caa

23

SEQ ID NO: 18

Length: 23

Type: DNA

Organism: Artificial Sequence

Feature:

Other Information: Description of Artificial

Sequence:Oligonucleotide

which binds non-converted human GST-Pi gene

Sequence: 18

gggcctaggg agtaaacaga cag

23

SEQ ID NO: 19

Length: 25

Type: DNA

Organism: Artificial Sequence

Feature:

Other Information: Description of Artificial

Sequence:Oligonucleotide

which binds human GST-Pi gene

Sequence: 19

6/17

cctttccctc tttcccarrrt cccca

25

SEQ ID NO: 20

Length: 25

Type: DNA

Organism: Artificial Sequence

Feature:

Other Information: Description of Artificial

Sequence:Oligonucleotide

which binds bisulfite-converted human GST-Pi gene

Sequence: 20

tttggtatattt tttttcgggt tttag

25

SEQ ID NO: 21

Length: 25

Type: DNA

Organism: Artificial Sequence

Feature:

Other Information: Description of Artificial

Sequence:Oligonucleotide

which binds non-converted human GST-Pi gene

Sequence: 21

cttgcatcc tcccccgggc tccag

25

SEQ ID NO: 22

Length: 26

Type: DNA

Organism: Artificial Sequence

Feature:

7/17

Other Information: Description of Artificial  
Sequence: Oligonucleotide  
which binds human GST-Pi gene

Sequence: 22  
ggyaggggaag ggaggyaggg gytggg 26

SEQ ID NO: 23  
Length: 31  
Type: DNA  
Organism: Homo sapiens

Sequence: 23  
ttatgtaata aatttgtata ttttgtatat g 31

SEQ ID NO: 24  
Length: 25  
Type: DNA  
Organism: Homo sapiens

Sequence: 24  
tgtagattat ttaaggtag gagtt 25

SEQ ID NO: 25  
Length: 27  
Type: DNA  
Organism: Homo sapiens

Sequence: 25  
aaacctaataa aataaacaaa caacaaa 27

SEQ ID NO: 26  
Length: 29  
Type: DNA  
Organism: Homo sapiens

8/17

Sequence: 26  
aaaaaacctt tccctctttc ccaaattccc 29

SEQ ID NO: 27  
Length: 27  
Type: DNA  
Organism: Homo sapiens

Sequence: 27  
tttggtggtt gtttattttt taggttt 27

SEQ ID NO: 28  
Length: 26  
Type: DNA  
Organism: Homo sapiens

Sequence: 28  
gggatttggt aaagagggaa aggttt 26

SEQ ID NO: 29  
Length: 24  
Type: DNA  
Organism: Homo sapiens

Sequence: 29  
actaaaaact ctaaacccca tccc 24

SEQ ID NO: 30  
Length: 24  
Type: DNA  
Organism: Homo sapiens

Sequence: 30  
aacctaatac taccttaacc ccat 24

9/17

SEQ ID NO: 31  
Length: 33  
Type: DNA  
Organism: Homo sapiens

Sequence: 31  
aatcctcttc ctactatcta ttactccct aaa 33

SEQ ID NO: 32  
Length: 29  
Type: DNA  
Organism: Homo sapiens

Sequence: 32  
aaaaccta aaataaaaaaa aaacttccc 29

SEQ ID NO: 33  
Length: 29  
Type: DNA  
Organism: Homo sapiens

Sequence: 33  
ttggttttat gttgggagtt ttgagtttt 29

SEQ ID NO: 34  
Length: 29  
Type: DNA  
Organism: Homo sapiens

Sequence: 34  
ttttgtgggg agttgggggttgatgttgt 29

SEQ ID NO: 35  
Length: 29  
Type: DNA  
Organism: Homo sapiens

10/17

Sequence: 35  
ggtttagagt ttttagtatg gggttaatt 29

SEQ ID NO: 36  
Length: 20  
Type: DNA  
Organism: Homo sapiens

Sequence: 36  
tagtattagg ttagggtttt 20

SEQ ID NO: 37  
Length: 29  
Type: DNA  
Organism: Homo sapiens

Sequence: 37  
aactctaacc ctaatctacc aacaacata 29

SEQ ID NO: 38  
Length: 29  
Type: DNA  
Organism: Homo sapiens

Sequence: 38  
caaaaaactt taaataaacc ctctacca 29

SEQ ID NO: 39  
Length: 32  
Type: DNA  
Organism: Homo sapiens

Sequence: 39  
gttttggtggt taggttggtt ttaggtggt ag 32



11/17

SEQ ID NO: 40  
Length: 30  
Type: DNA  
Organism: Homo sapiens

Sequence: 40  
gttttgagta tttgttgtgt ggtagttttt 30

SEQ ID NO: 41  
Length: 30  
Type: DNA  
Organism: Homo sapiens

Sequence: 41  
ttaatatataaa taataaaaaat atattttacaa 30

SEQ ID NO: 42  
Length: 34  
Type: DNA  
Organism: Homo sapiens

Sequence: 42  
caacccccaa tacccaaccc taatacaaat actc 34

SEQ ID NO: 43  
Length: 26  
Type: DNA  
Organism: Homo sapiens

Sequence: 43  
ggtttttagtt tttggttggtt tggatg 26

SEQ ID NO: 44  
Length: 26  
Type: DNA

12/17

Organism: Homo sapiens

Sequence: 44

tttttttggt tttagtatat gtgggg

26

SEQ ID NO: 45

Length: 30

Type: DNA

Organism: Homo sapiens

Sequence: 45

ataactaaaa aactattttc taatcctcta

30

SEQ ID NO: 46

Length: 29

Type: DNA

Organism: Homo sapiens

Sequence: 46

ccaaactaaa aactccaaaa aaccactaa

29

SEQ ID NO: 47

Length: 38

Type: DNA

Organism: Artificial Sequence

Feature:

Other Information: Description of Artificial Sequence: M13-human  
GST-Pi oligonucleotide

Sequence: 47

tgtaaaacga cggccagtgg gatttgggaa agagggaa

38

SEQ ID NO: 48

Length: 38

Type: DNA

13/17

Organism: Artificial Sequence

Feature:

Other Information: Description of Artificial Sequence: M13-human  
GST-Pi oligonucleotide

Sequence: 48

tgtaaaacga cggccagttg ttgggagttt tgagtttt 38

SEQ ID NO: 49

Length: 31

Type: DNA

Organism: Artificial Sequence

Feature:

Other Information: Description of Artificial Sequence: M13-human  
GST-Pi oligonucleotide

Sequence: 49

tgtaaaacga cggccagtta gtattaggtt a 31

SEQ ID NO: 50

Length: 37

Type: DNA

Organism: Artificial Sequence

Feature:

Other Information: Description of Artificial Sequence: M13-human  
GST-Pi oligonucleotide

Sequence: 50

tgtaaaacga cggccagtgt tttgagtatt tgttgtg 37

SEQ ID NO: 51

Length: 35

Type: DNA

14/17

Organism: Artificial Sequence

Feature:

Other Information: Description of Artificial Sequence: M13-human  
GST-Pi oligonucleotide

Sequence: 51

tgtaaaacga cggccagtgt ttttagtata tgtgg 35

SEQ ID NO: 52

Length: 499

Type: DNA

Organism: Homo sapiens

Sequence: 52

tgcagatcac ctaagggtcag gagttcgaga ccagcccggc caacatggtg aaaccccgtc 60  
tctactaaaa atacaaaaat cagccagatg tggcacgcac ctataattcc acctactcgg 120  
gaggctgaag cagaattgct tgaacccgag aggcggaggt tgcagtgagc cgccgagatc 180  
gcgccactgc actccagcct gggccacagc gtgagactac gtcataaaat aaaataaaat 240  
aacacaaaaat aaaataaaat aaaataaaat aaaataaaat aataaaataa aataaaataa 300  
aataaaataa aataaaataa agcaatttcc tttcctctaa gcggcctcca cccctctccc 360  
ctgccctgtg aagcgggtgt gcaagctccg ggatcgcagc ggtcttaggg aatttcccc 420  
cgcgatgtcc cggcgcgcca gttcgctgcg cacacttcgc tgcggtcctc ttcctgctgt 480  
ctgtttactc cctaggccc 499

SEQ ID NO: 53

Length: 316

Type: DNA

Organism: Homo sapiens

Sequence: 53

gggacctggg aaagagggaa aggcttcccc ggccagctgc gcggcgactc cggggactcc 60  
agggcgcccc tctgcggccg acgcccgggg tgcagcggcc gccggggctg gggccggcgg 120  
gagtcgcggg gacctccag aagagcggcc ggcgcctga ctcagcactg gggcggagcg 180  
gggcgggacc acccttataa ggctcggagg ccgcgaggcc ttcgctggag tttcgccgcc 240  
gcagtcttcg ccaccagtga gtacgcgcgg ccgcgctccc cggggatggg gctcagagct 300

15/17

cccagcatgg ggccaa

316

SEQ ID NO: 54

Length: 603

Type: DNA

Organism: Homo sapiens

Sequence: 54

```
cagcatcagg cccgggctcc cggcagggct cctcgccac ctcgagaccc gggacggggg 60
cctaggggac ccaggacgtc ccagtgccg ttagcggctt tcagggggcc cggagcgct 120
cggggaggga tgggaccccg ggggcgggga gggggggcag gctgcgctca ccgcgccttg 180
gcatectccc ccgggtcca gcaaactttt ctttgttcgc tgcagtgcg ccctacaccg 240
tggtctatct cccagttcga ggtaggagca tgtgtctggc agggaaggga ggcaggggct 300
ggggctgcag cccacagccc ctcgcccacc cggagagatc cgaacccct tatccctccg 360
tcgtgtggct tttaccccg gcctccttcc tgttccccgc ctctcccgcc atgctgtctc 420
cccgccccag tgttgtgtga aatcttcgga ggaacctgtt tacctgttcc ctccctgcac 480
tcctgacccc tccccgggtt gctgcgaggc ggagtcggcc cgggtccccc atctcgtact 540
tctccctccc cgcaggccgc tgcgcggccc tgcgcatgct gctggcagat cagggccaga 600
gct 603
```

SEQ ID NO: 55

Length: 266

Type: DNA

Organism: Homo sapiens

Sequence: 55

```
gctctgagca cctgctgtgt ggcagtctct catccttcca cgcacatcct cttccctcc 60
tcccaggctg gggctcacag acagccccct ggttgggcca tcccagtgga ctgtgtgttg 120
atcaggcgcc cagtcacgcy gcctgctccc ctccacccaa cccagggtct ctatgggaag 180
gaccagcagg aggcagccct ggtggacatg gtgaatgacg gcgtggagga cctccgctgc 240
aaatacatct ccctcatcta caccaa 266
```

SEQ ID NO: 56

Length: 287

Type: DNA

Organism: Homo sapiens

16/17

Sequence: 56

```
tccccctgct ctcagcatat gtggggcgcc tcagtgcccg gcccaagctc aaggccttcc 60
tggcctcccc tgagtacgtg aacctcccca tcaatggcaa cgggaaacag tgagggttgg 120
ggggactctg agcgggagggc agagtttgcc ttcctttctc caggaccaat aaaattttcta 180
agagagctac tatgagcact gtgtttcctg ggacggggct taggggttct cagcctcgag 240
gtcgggtgga gggcagagca gaggactaga aaacagctcc tccagca 287
```

SEQ ID NO: 57

Length: 524

Type: DNA

Organism: Homo sapiens

Sequence: 57

```
ataaaataaa ataaaataaa ataaagcaat ttcctttcct ctaagcggcc tccacccctc 60
tcccctgccc tgtgaagcgg gtgtgcaagc tccgggatcg cagcgggtctt agggaatttc 120
cccccgcat gtcccgggcg gccagttcgc tgcgcacact tcgctgcggt cctcttctctg 180
ctgtctgttt actccctagg ccccgctggg gacctgggaa agagggaaag gcttcccccg 240
ccagctgctg ggcgactccg gggactccag ggcgcccctc tgcggccgac gcccggggtg 300
cagcgggcgc cggggctggg gccggcggga gtccgcggga ccctccagaa gagcgggcgg 360
cgccgtgact cagcactggg gcggagcggg gcgggaccac cttataagg ctgggagggc 420
gcgaggcctt cgctggagtt tcgccgccgc agtcttcgcc accagtgagt acgcgcggcc 480
cgcgccccg gggatggggc tcagagctcc cagcatgggg ccaa 524
```

SEQ ID NO: 58

Length: 524

Type: DNA

Organism: Homo sapiens

Sequence: 58

```
ataaaataaa ataaaataaa ataaagtaat tttttttttt ttaagtgggt tttatttttt 60
ttttttgttt tgtgaagtgg gtgtgtaagt tttgggattg tagtggtttt agggaatttt 120
tttttgtgat gttttggtgt gttagtttgt tgtgtatatt ttgttgtggt tttttttttg 180
ttgtttgttt attttttagg ttttgttggg gatttgggaa agagggaaag gttttttttg 240
ttagttgtgt ggtgattttg gggatttttag ggtgtttttt tgtggttgat gtttgggggtg 300
tagtggttgt tggggttggg gttggtggga gtttgtggga ttttttagaa gagtgggttg 360
```

17/17

```
tggtgtgatt tagtattggg gtggagtggg gtgggattat tttataagg tttggagggt 420
gtgagggttt tggtggagtt ttgttgttgt agtttttgtt attagtgagt atgtgtgggt 480
tgtgtttttg gggatggggt ttagagtttt tagtatgggg ttaa 524
```

SEQ ID NO: 59

Length: 524

Type: DNA

Organism: Homo sapiens

Sequence: 59

```
ataaaataaa ataaaataaa ataaagtaat tttttttttt ttaagcggtt tttatttttt 60
ttttttgttt tgtgaagcgg gtgtgtaagt ttcgggatcg tagcggtttt agggaatttt 120
ttttcgcgat gtttcggcgc gttagttcgt tgcgtatatt tcgttgcggt tttttttttg 180
ttgtttgttt attttttagg tttcgttggg gatttgggaa agagggaaag gttttttcgg 240
ttagttgcgc ggcgatttcg gggatttttag ggcgtttttt tgcggtcgac gttcgggggtg 300
tagcggtcgt cgggggttggg gtcggcgga gttcgcgga ttttttagaa gagcggtcgg 360
cgtcgtgatt tagtattggg gcggagcggg gcgggattat tttataagg ttcggaggtc 420
gcgagggttt cgttggagtt tcgtcgtcgt agttttcgtt attagtgagt acgcgcgggt 480
cgcgttttcg gggatggggt ttagagtttt tagtatgggg ttaa 524
```